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Dark chocolate intake buffers stress reactivity in humans

Brief title: Dark chocolate and stress reactivity

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ABBREVIATIONS

ACTH - adrenocorticotrophic hormone

CVD – cardiovascular disease

EDTA - ethylenediaminetetraacetic acid

HPA axis - hypothalamus-pituitary-adrenal axis

HPLC - high-pressure liquid chromatography

MAP – mean arterial blood pressure, calculated by the formula $(2/3 \times \text{diastolic blood pressure (BP)}) + (1/3 \times \text{systolic BP})$ from two blood pressure measurements under resting conditions

PASA – Primary Appraisal Secondary Appraisal Scale, Gaab et al. 2005,

Psychoneuroendocrinology 30(6):599-610; the PASA Stress Index combines primary (i.e., the judgment about the significance of an event as stressful, positive, controllable, challenging, or irrelevant) and secondary appraisal (i.e., the assessment of available coping resources and options when faced with a stressor) providing an integrated measure of transactional stress perception with higher scores representing higher anticipatory cognitive stress appraisal

SEM – standard error of mean

SNS - sympathetic nervous system

TSST – Trier Social Stress Test, Kirschbaum et al. 1993, Neurobiology 28(1-2):76-81.

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HPLC plasma analyses were performed in the Laboratory for Stress Monitoring (Göttingen, Germany), ACTH was analyzed by Cytolab (Regensdorf, Switzerland), and cortisol concentrations were determined in the biochemical laboratory at the Institute of Psychology of the University of Zurich, Switzerland.

MANUSCRIPT TEXT

Dark chocolate consumption substantially lowers cardiovascular mortality due to the high content of polyphenolic flavonoids (1), but underlying mechanisms remain unclear. Psychosocial stress is a risk factor supposed to promote CVD by inducing HPA axis and SNS stress responses implicated to increase CVD risk, either by direct effects and/or by inducing adverse changes in intermediate biological risk factors (2). Animal studies suggest that flavonoid administration may protect from adverse stress effects by reducing stress responses including HPA axis activation (e.g.3). One human study assessed in healthy men endocrine stress reactivity after 6 weeks of consuming either flavonoid containing tea or flavonoid-free placebo tea and found a faster decline of cortisol levels after a moderate mental stress task in the active tea group (4). Here, we investigate whether a single administration of dark chocolate buffers endocrine reactivity to acute psychosocial stress in healthy men and whether this effect relates to plasma levels of the flavonoid epicatechin. Moreover, we wanted to distinguish whether this effect would be peripheral (by measuring the adrenal gland hormones cortisol and epinephrine) or more central (by assessing ACTH, norepinephrine, and cognitive stress appraisal).

In a placebo-controlled between-subjects design healthy, medication-free, non-smoking men (20-50years) were age-matched assigned to the experimental dark chocolate group (N=31) or the placebo control group (N=34). The dark chocolate ("Noir 72%", Chocolat Frey AG, Buchs AG) contained 281kcal and 125mg epicatechin per serving of 50g. The optically identical placebo chocolate (310.5 kcal and 0mg epicatechin per 50g serving) was a flavonoid-free white chocolate that was dyed and flavored to match the color, appearance, and smell of the dark chocolate. After a standardized breakfast at 10:00am a venous catheter was inserted at 10:45am followed 45min later by the first saliva and blood sampling with subsequent administration of 50g of dark or placebo chocolate. Subjects underwent the psychosocial stressor 2h after chocolate ingestion, when we expected plasma

flavonoid levels to peak. We applied the TSST combining a 3-min preparation phase after a short introduction, a 5-min mock job interview, and a 5-min mental arithmetic task in front of an audience.

As stress hormones secreted from the adrenal gland and thus in the periphery only we measured the HPA-axis hormone cortisol (secreted by the adrenal cortex) and the SNS-hormone epinephrine (secreted by the adrenal medulla). As hormones indicating a more central stress effect we measured the HPA-axis hormone ACTH secreted by the anterior pituitary and the SNS-hormone norepinephrine released both as neurotransmitter from sympathetic nerve endings and to a smaller extent as stress hormone from the adrenal medulla. Saliva (Salivette, Sarstedt, Rommelsdorf, Germany) and blood samples were collected before chocolate consumption and immediately before TSST. Additional saliva samples were collected immediately after and up to 60min after stress cessation. Additional blood samples were obtained immediately and 10min after TSST (epinephrine, norepinephrine, ACTH), as well as 60min (ACTH) and 120min (epicatechin) after stress cessation. Blood was drawn into EDTA-coated monovettes (Sarstedt, Numbrecht, Germany) and immediately centrifuged for 10min at 2,000g and 4°C; plasma was stored at –80°C until analysis. Salivettes were stored at –20°C until biochemical analysis. Plasma ACTH concentrations were determined with a beads immunoassay (Human-Pituitary-Bead-Panel-1, Millipore, Zug, Switzerland) on a Guava EasyCyte flow cytometer (Millipore, Zug, Switzerland). Salivary cortisol was analyzed with a competitive chemiluminescence immunoassay (LIA, IBL Hamburg, Germany). Plasma epinephrine, norepinephrine, and epicatechin levels were quantified by HPLC using electrochemical detection. Intra- and inter-assay variabilities were below 10%. As a psychological stress measure we assessed anticipatory cognitive stress appraisal using the PASA-questionnaire.

Univariate ANOVAs (unit,dark chocolate group:mean \pm SEM/placebo group:mean \pm SEM) revealed that, the groups significantly differed in epicatechin plasma

levels before (ng/ml, 40.5 ± 2.9 , $p < .001$) and 120min (ng/ml, 16.7 ± 1.1 , $p < .001$) after stress. Moreover, there were no group differences in stress hormone levels before chocolate consumption (cortisol: nmol/l, 10.1 ± 1.5 / 9.9 ± 1.2 , $p = .90$; ACTH: pg/ml, 6.9 ± 1.7 / 8.6 ± 3.2 , $p = .66$; epinephrine: pg/ml, 26.6 ± 3.6 / 19.9 ± 2.1 , $p = .33$; norepinephrine: pg/ml, 397.4 ± 25.7 / 446.1 ± 33.9 , $p = .26$), age (years, 34.5 ± 1.6 / 36.8 ± 1.5 , $p = .30$), BMI (kg/m^2 , 25.0 ± 0.8 / 25.2 ± 0.7 , $p = .84$), MAP (mmHg, 89.6 ± 1.8 / 91.3 ± 1.6 , $p = .48$), or stress appraisal (PASA-Stress-Index, $-.79 \pm .58$ / $-.45 \pm .45$, $p = .64$). To test whether dark chocolate consumption induces changes in stress hormone reactivity to acute psychosocial stress, we calculated general linear models with repeated measures, while controlling for the pre-chocolate baseline of the respective stress hormone as covariate. Across all subjects, the TSST induced significant increases in cortisol, ACTH, epinephrine, and norepinephrine (p 's $< .001$). The dark chocolate group showed a significantly blunted cortisol (interaction group-by-stress $F(2.5/154.8) = 7.47$, $p < .001$, $\eta^2 = .108$, $f = .35$, Fig. 1A) and epinephrine (interaction group-by-stress $F(1.7/101.0) = 4.34$, $p = .021$, $\eta^2 = .066$, $f = .27$, Fig. 1B) reactivity to psychosocial stress as compared to the placebo group. Additional controlling for age, BMI, and MAP did not significantly change these results (interaction group-by-stress cortisol: $F(2.6/155.6) = 6.59$, $p = .001$, $\eta^2 = .100$, $f = .33$; epinephrine: $F(1.8/101.8) = 4.06$, $p = .025$, $\eta^2 = .065$, $f = .26$). There were no group differences in terms of ACTH or norepinephrine stress reactivity (p 's $> .26$). To test whether epicatechin plasma levels prior to the TSST would predict subsequent physiological stress reactivity, we recalculated the previous general linear models but entered as independent variable pre-stress epicatechin plasma levels instead of group. Higher epicatechin plasma levels significantly related to lower stress reactivity of the adrenal gland hormones cortisol (interaction group-by-stress: $F(2.4/143.5) = 3.46$, $p = .027$, $\eta^2 = .054$, $f = .24$) and epinephrine (interaction group-by-stress $F(1.7/99.2) = 3.36$, $p = .047$, $\eta^2 = .053$, $f = .24$) across both subject groups, also independent of age, BMI, and MAP (interaction group-by-stress

cortisol: $F(2.5/145.3)=3.24, p=.032, \eta^2=.053, f=.24$; epinephrine: $F(1.8/99.9)=3.62, p=.036, \eta^2=.060, f=.25$). There were no associations of epicatechin levels with ACTH or norepinephrine stress reactivity (p 's $>.27$).

Our findings indicate that acute flavonoid-rich dark chocolate intake buffers endocrine stress reactivity on the level of the adrenal gland suggesting a peripheral stress-protective effect of dark chocolate consumption, particularly, since in the chocolate group the unaffected ACTH stress response did not result in correspondingly high cortisol secretion. While it is unclear whether epicatechin can access the human brain at levels sufficiently high to modify central nervous processes, inhibitory peripheral effects of dietary flavonoids on the biosynthesis and secretion of cortisol and catecholamines seem plausible (e.g. (5)). Strengths of our study include the use of a unique placebo chocolate and of a well-validated stressor. Future research is needed to determine mediating mechanisms, clinical relevance, long-term health consequences, and generalizability to chronic stress exposure and populations other than healthy men.

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Legend to Figure 1

Values are means \pm SEM. General linear models with repeated measures of cortisol (Figure 1A) and epinephrine (Figure 1B) as dependent variables and chocolate group as the independent variable revealed that stress reactivity of both adrenal hormones was blunted in the dark chocolate group as compared to the placebo group (cortisol: $F(2.5/154.8)=7.47$, $p<.001$; epinephrine: $F(1.7/101.0)=4.34$, $p=.021$). Baseline levels of the respective parameter were controlled.

Figure 1. Physiological reactivity to psychosocial stress (Trier Social Stress Test, TSST) in the dark chocolate and the placebo chocolate group.



